Impact of predicted end-of-century temperature on calcification and tissue color of the temperate coral, *Oculina arbuscula*

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Abstract

Rising ocean temperatures affect coral calcification and symbiont density. Significant water warming has been correlated with reduced calcification and loss of photosynthetic symbionts, known as coral bleaching. Such changes negatively impact corals as they are no longer able to grow or derive nutrients from their symbionts. Traditional methods of measuring bleaching, such as quantifying symbiont density and chlorophyll-*a* content, are destructive to corals and time consuming. Thus, non-invasive and non-destructive color analysis techniques have been shown to accurately predict symbiont density in several Indo-Pacific coral species. However, little research has been done to determine the efficacy of such color analysis techniques on temperate coral species. This study investigated the impact of water temperature on net calcification of the temperate coral *Oculina arbuscula* and used red-green-blue (RGB) color analysis to assess associated changes in tissue color within this species. Corals were reared under three temperature treatments: an *in situ* temperature control from the coral harvesting site during the month of October and two elevated temperature profiles reflecting end-of-century sea surface temperature models, *in situ* + 4°C (RCP 6.0) and *in situ* + 6°C (RCP 8.5). We measured the buoyant weight of each coral at the start and conclusion of the experiment to measure net calcification. We found that temperature did not have a significant impact on the net calcification of *O. arbuscula* across treatments during the 28-day experimental period. For color analysis, we took weekly photographs of each coral and extracted color intensity values from the images. We found that prolonged exposure to thermal stress in the *in situ* + 4°C (RCP 6.0) and *in situ* + 6°C (RCP 8.5) treatments resulted in loss of tissue color. As tissue color is correlated with symbiont density, these findings suggest that temperature impacts symbiont density and that RGB color analysis can detect thermally-induced changes in symbiont density of *O. arbuscula*. As anthropogenic

climate change continues to increase ocean temperatures and stress corals, rapid and non-destructive methods for assessing coral health, such as color analysis, will become ever more important to predicting the futures of coral species.

Introduction

Global climate change continues to impact ocean environments by increasing the partial pressure of CO_2 (pCO_2), increasing water temperature, and decreasing pH. Global atmospheric carbon dioxide has increased from approximately 280 ppm during the Industrial Revolution to present-day values exceeding 400 ppm (Doney et al., 2009). Increased atmospheric pCO_2 has resulted in an increase in global sea surface temperature (SST) of 0.11°C per decade from 1971 through 2010 (Rhein et al., 2013). These trends in increasing global SST are predicted to continue through the end of the century (Rhein et al., 2013). Representative concentration pathways (RCP) are models that predict global environmental changes based on projected greenhouse gas (GHG) emissions and land use (Pachauri et al., 2015). RCP projections are based on human action taken to reduce anthropogenic emissions, ranging from a strict mitigation scenario (RCP 2.6) to a scenario with very high GHG emissions (RCP 8.5) (Pachauri et al., 2015). RCP 6.0 and RCP 8.5 model scenarios with no efforts to reduce GHG emissions (Pachauri et al., 2015). RCP 6.0 and RCP 8.5 project end-of-century global increases in SST of 3-4°C and 5-6°C, respectively (Hoegh-Guldberg et al., 2014).

Increasing SST especially affects corals, as significant warming has been correlated with reduced calcification and loss of photosynthetic pigment (Jokiel & Coles, 1977). Hard corals have calcium carbonate skeletons, and their ability to produce calcium carbonate is affected by water temperature (Castillo et al., 2014; Jokiel & Coles, 1977; Miller, 1995). Calcification of many

coral species has been shown to exhibit a parabolic response to increasing water temperature (Castillo et al., 2014; Jokiel & Coles, 1977). One such study found that calcification of the Caribbean coral, *Siderastrea siderea*, increased with moderate warming matching ambient summer water temperatures but declined under the highest thermal conditions (Castillo et al., 2014). Similarly, three common species of Hawaiian reef corals exhibited maximum skeletal growth at summer water temperatures, but declining growth and even mortality with prolonged exposure to water temperatures exceeding natural summer conditions (Jokiel & Coles, 1977).

Increasing SST also impacts corals' photosynthetic symbionts, on which they rely for a major source of nutrition. Rising temperatures stress corals and cause symbionts to be expelled from their coral host, which is defined as coral bleaching (D'Croz et al., 2001). Although bleaching does not initially result in coral mortality, the event makes corals extremely vulnerable to another stressor event (Bove et al., 2019). As SST continues to rise, corals are expected to suffer higher rates of bleaching; therefore, a method that accurately quantifies coral symbionts and bleaching is essential to assessing coral health (Chow et al., 2016; D'Croz et al., 2001). Current methods to measure bleaching, such as the analysis of symbiont density and chlorophyll-*a* pigment, are time consuming, invasive, and result in coral mortality. However, alternative techniques have been proposed to quantify symbionts from photographs: a method that is efficient, non-invasive, accurate, and can even be conducted with corals in the field (Chow et al., 2016; Winters et al., 2009). In one study, chlorophyll density of *Stylophora pistillata* was found to be highly negatively correlated with the intensity of the red color channel from color analysis (Winters et al., 2009). Another study found chlorophyll-*a* concentration and symbiont density of *Goniopora lobata* to be negatively correlated with estimated percent whiteness from photographic analysis (Chow et al., 2016). The red-green-blue (RGB) color analysis technique used in this experiment

involves taking overhead-view photos of coral fragments (ramets) and color-correcting the images using a true white reference. A MATLAB program can then be used to quantify the intensity of red, green and blue color channels of each ramet. Color intensity can then be compared to symbiont count and chlorophyll-*a* data from standard methods to assess the accuracy of the RGB technique for measuring tissue color and coral bleaching.

This study investigated the impacts of elevated water temperature on the calcification and tissue color of the temperate coral, *Oculina arbuscula*. *O. arbuscula* is a facultative symbiotic coral that inhabits the coastal waters of the Mid-Atlantic United States and provides habitat for hundreds of ecologically and economically important species (Miller, 1995; Deaton et al., 2010). Corals are mixotrophs, meaning they derive energy from their algal symbionts and through heterotrophic feeding on dissolved organic matter (Smith et al., 2016). *O. arbuscula* is a somewhat unique species as they naturally exist with and without symbionts (Miller, 1995). Unlike many tropical coral species, white colonies of *O. arbuscula* are not necessarily bleached. Some temperate corals, such as *O. arbuscula*, can dramatically increase their reliance on heterotrophy when under stress (Smith et al., 2016). Corals used in this experiment ranged from almost entirely white and aposymbiotic to dark reddish-brown colonies with lots of symbionts. In addition to being able to tolerate a wide range of light conditions, temperate corals have been shown to tolerate temperature and salinity fluctuations (Miller, 1995). The ability of temperate corals to adapt to varying conditions suggest these corals will be able to better withstand environmental changes resulting from climate change (Miller, 1995). Thus, by studying the adaptable species *O. arbuscula*, we can begin to understand what makes these corals so resilient in the face of environmental change to better inform coral conservation decisions.

Methods

Coral collection and maintenance

Seven colonies of *O. arbuscula* were harvested from 3-6 meter depths at the Radio Island Jetty in Beaufort, North Carolina (34° 42’ 28.45” N, 76° 40’ 47.06” W) on September 24, 2020 (Figure 1). Colonies harvested were at least 10 m apart to ensure they were genetically different. The collected colonies varied in color, from white to reddish-brown, suggesting a spectrum of symbiont density. The colonies were loosely wrapped in seawater-soaked paper towels and transported to the Aquarium Research Center at the University of North Carolina at Chapel Hill. The colonies were placed in two 500 L recirculating holding tanks at 24° C to match the *in situ* temperature at the time of coral collection. Holding tanks were maintained at a salinity of 35 to match water samples collected at-depth when harvesting corals. Deionized water and Instant Ocean Sea Salt were used to make the saltwater mixes (Supplementary Methods). Flow rate and temperature were controlled by pumps, chillers and heaters (Supplementary Methods). Photosynthetically active radiation, pH and nitrate levels were kept within natural environmental ranges (Supplementary Methods).

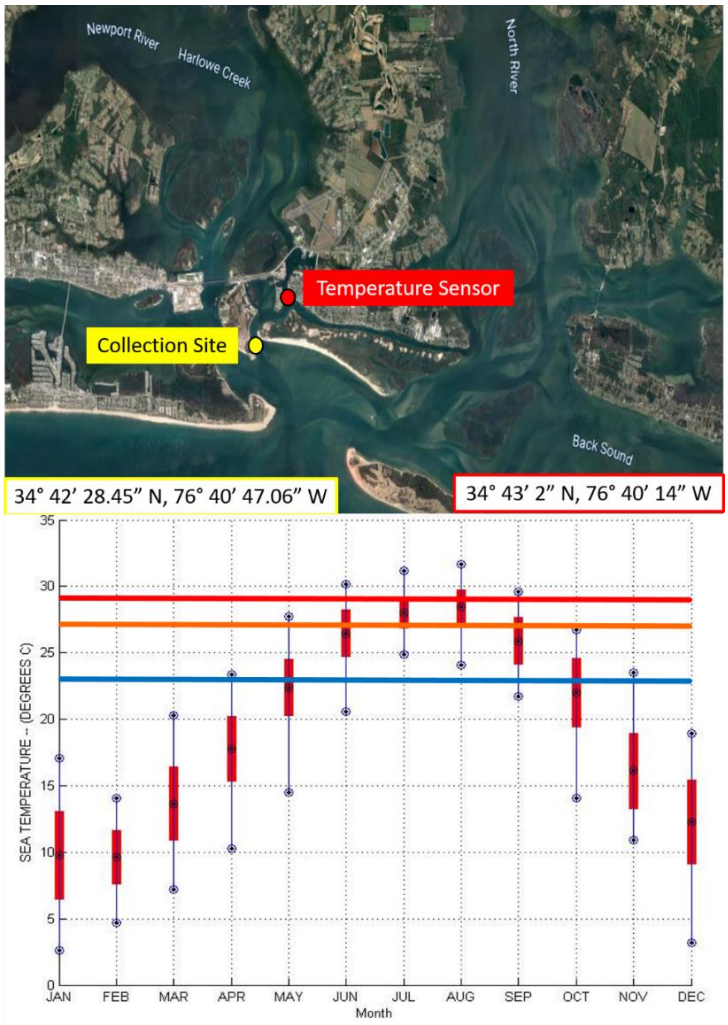

Figure 1. Location and mean temperatures at the collection site. Map showing the location where *O. arbuscula* colonies were collected at Radio Island Jetty, NC (yellow dot) and location of NOAA buoy BFTN7 where *in situ* temperatures were recorded (red dot). The graph shows the average monthly SST for 2005-2012 from NOAA buoy BFTN7. Horizontal lines show the average temperature for the three temperature treatments: *in situ* (blue), *in situ* + 4°C/RCP 6.0 (orange) and *in situ* + 6°C/RCP 8.5 (red). (Figure adapted from NOAA Station BFTN7 Climactic Summary Plots for sea temperature).

Coral recovery and temperature acclimation

Using a diamond-embedded band saw, each colony was sectioned into twelve approximately 5 cm^3 ramets. Three ramets of each colony were sacrificed to measure initial symbiont density. The remaining nine ramets of each colony were randomly assigned to a temperature treatment and used in the experiment (Supplementary Figure 1). Each experimental ramet was fixed to a labeled, sterile petri dish using cyanoacrylate. The experimental ramets were returned to the holding tanks to recover for five days. The ramets were placed randomly within their respective experimental tank to reduce bias against optimal flow rates and light conditions within the tanks. Nine 38 L experimental tanks were divided into three identical experimental systems, with three

tanks per system (Supplementary Figure 2). Three tanks were maintained at the *in situ* temperature from the collection site (control), three were maintained at *in situ* temperature + 4°C (RCP 6.0), and three were maintained at *in situ* temperature + 6°C (RCP 8.5) (Supplementary Methods). *In situ* temperatures were derived from the average weekly temperatures reported by NOAA buoy (station BFTN7) (Figure 1). The ramets were allowed to recover for five more days in the experimental tanks, for a total of ten days after sectioning. The initial buoyant weight of each ramet was measured at the conclusion of the recovery period (precision = 0.0001g; Mettler-Toledo; Davies, 1989). Over the following four days, the water temperature in the experimental tanks was slowly adjusted to the respective temperature treatment. The buoyant weight of each ramet was measured at the conclusion of the acclimation period. The corals were removed from the tanks and photographed using a digital camera (Canon EOS Rebel T6i) against the same white background. These photographs were used for initial intensity values in color analysis. The camera was mounted on a tripod at a 90° angle 20 cm above the background.

Experimental period

The experimental period lasted 28 days. At the beginning of each week, the temperature in the tanks was adjusted to reflect the average *in situ* temperature of the previous week recorded by NOAA buoy (station BFTN7) to account for natural temperature variation. Photographs of each ramet were taken at the start of each week and at the end of the experimental period. The ramets were placed back in the tanks at randomized positions each week after they were photographed. Corals were fed one-day-old *Artemia sp. nauplii* (250 count/L) daily at least one hour after the actinic lights turned off to simulate crepuscular feeding (Supplementary Methods). This feeding concentration was determined from the average copepod abundance measured in Beaufort NC, near the coral collection site (Fulton, 1984). The buoyant weight of each ramet was measured at

the conclusion of the experimental period. Each ramet was wrapped in tin foil and frozen at -80°C to preserve samples until tissue analysis was performed.

RGB Color analysis

Photographs of the corals were taken throughout the experiment to be used in color analysis. To standardize the photos in terms of color balance, a custom macro in Python (python.adjust) was run. A region 25 x 25 pixels of the white background of each photo was selected and converted to true white, which normalized the image color. A custom-made macro in MATLAB (Coral Color Intensity Analysis Utility) was used to extract red, green and blue intensity values from the color-balanced photos. Twenty 25 x 25 pixel points were selected manually at random, while avoiding shadows and glare. Average values for red, green and blue intensity of each photo were recorded.

Statistical analysis

Statistical analysis was performed in R Studio (version 3.0.1). Analysis of variance (one-way ANOVA, function anova()) was used to determine the effect of temperature regime on net calcification and the effect of colony on color intensity. Post hoc Tukey's HSD tests were used to evaluate the significance of each pairwise comparison ($\alpha = 0.05$).

Results

Temperature did not significantly affect net calcification across treatments

Predicting coral growth in response to increasing water temperature is essential to assess the future of coral communities in the face of anthropogenic climate change. To determine if water temperature impacted net calcification of *O. arbuscula*, buoyant weight measurements were

taken before and after the experimental period. We found that temperature did not have a significant impact on the net calcification of *O. arbuscula* across treatments during the 28-day experimental period (ANOVA p=0.1266) (Figure 2).

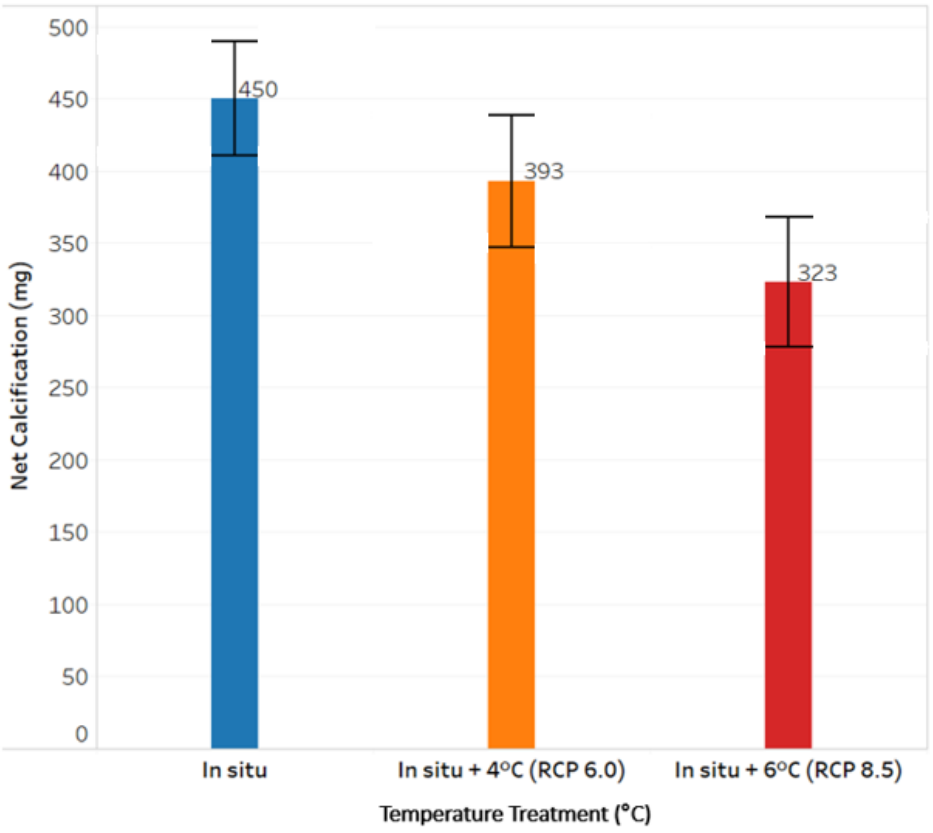

Figure 2. Net calcification measured by buoyant weights did not vary significantly across temperature treatments predicted by end-of-century RCP models during the 28-day experimental period. Mean values are labeled. Standard error bars are shown. N = 21 ramets per temperature treatment.

Colony-level differences in net calcification across treatments

Because there were obvious differences in the initial tissue color of the colonies used in this experiment which can affect calcification, we investigated colony-level calcification in response to water temperature. Water temperature influenced the net calcification of some colonies more than others. Colonies A, E, F and G exhibited lower net calcification in the *in situ* + 6°C (RCP 8.5) treatment than in the *in situ* temperature treatment. Net calcification of colonies B, C and D did not differ with water temperature (Figure 3).

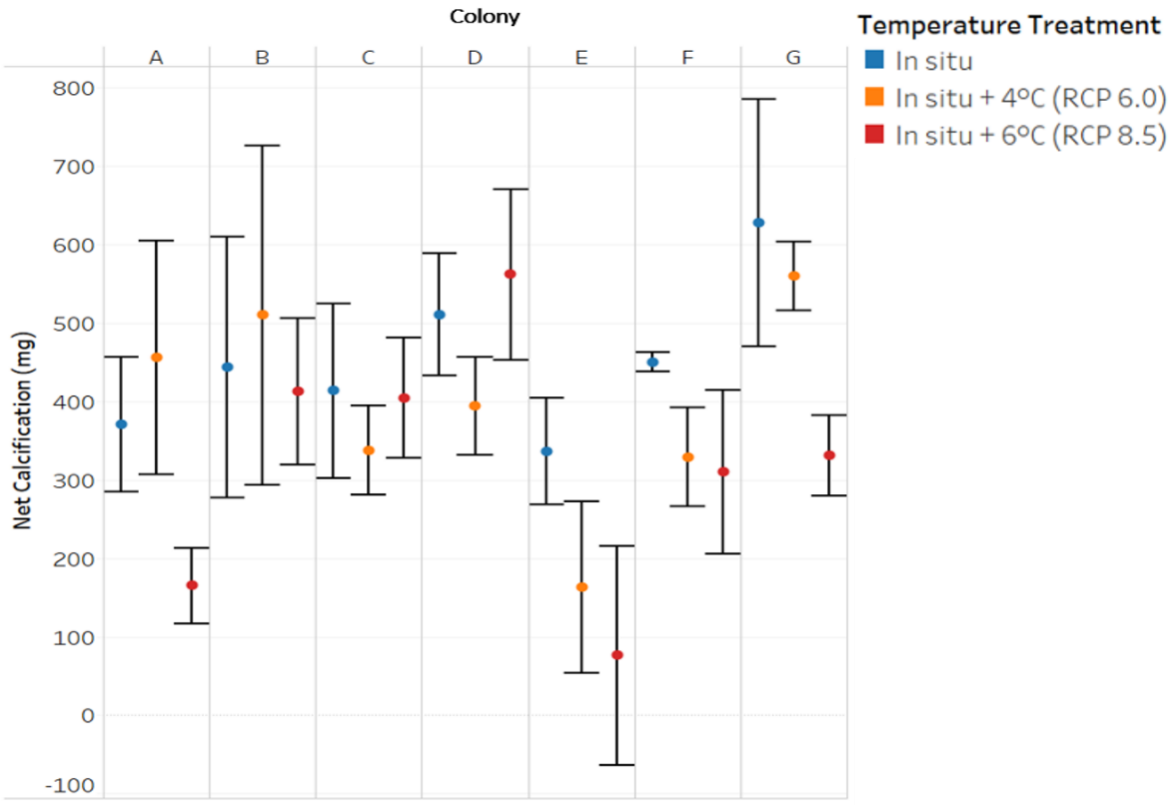

Figure 3. Temperature treatment variably affected colony net calcification measured by buoyant weights. Mean values are labeled. Standard error bars are shown. N = 9 ramets per colony.

Temperature treatment influenced color intensity

Analysis of coral tissue color can indicate the presence or absence of algal symbionts. To determine if water temperature affects coral color, intensity values of red, green and blue channels and the sum of all color channels were measured. As this technique used additive color mixing theory, low intensity values correlated with more colorful corals (Supplementary Figure 3). Change in color intensity over time was influenced by temperature treatment (Figure 4). Ramets in the *in situ* temperature treatment exhibited decreasing sum color intensity values throughout the experiment. Ramets in the *in situ* + 4°C (RCP 6.0) temperature treatment exhibited a steady decline in sum color intensity from Week 0 to Week 4, but increased during

the final week of the experiment. Ramets in the *in situ* + 6°C (RCP 8.5) temperature treatment showed the largest decline in sum color intensity from Week 0 to Week 1. Sum color intensity plateaued from Week 1 to Week 4 before increasing for the final week of the experiment. Intensity trends for red, green and blue color channels largely matched sum color intensity trends across all temperature treatments (Supplementary Figure 4).

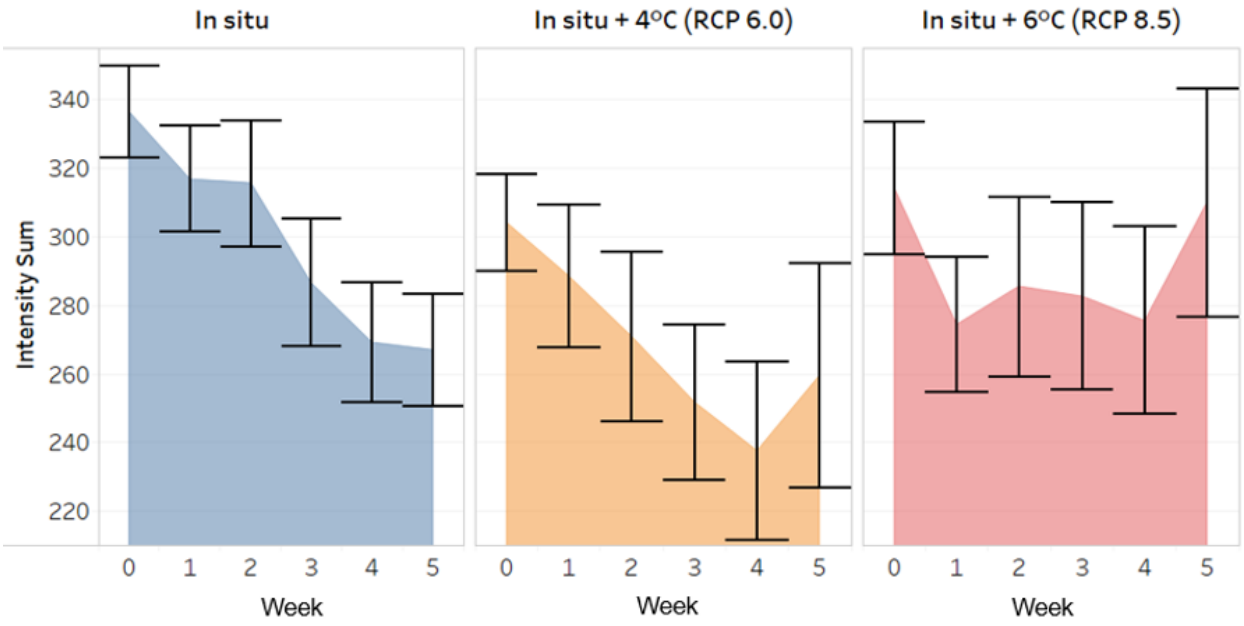

Figure 4. The effect of water temperature on intensity sum across all temperature treatments. Week 0 marks the acclimation period. Week 1 –Week 5 marks the duration of the experimental period. Standard error bars are shown. N = 24 ramets per temperature treatment for Week 0 and 21 ramets per temperature treatment for Week 1 - Week 5.

Discussion

Coral temperature tolerance maintains positive net calcification

Buoyant weight measurements showed that temperature did not have a significant impact on the net calcification of *O. arbuscula* across treatments during the 28-day experimental period. *O. arbuscula* is known to have a wide temperature tolerance, which likely contributed to similar net calcification values regardless of temperature treatment (Miller, 1995). Because temperature

treatments were based on *in situ* temperatures during the month of October, the elevated temperature treatments (*in situ* + 4°C/RCP 6.0 and *in situ* + 6°C/RCP 8.5) did not exceed July water temperatures ranges of the past decade (Figure 1). Thus, corals in this experiment were not exposed to water temperatures above the seasonal variation they experienced in their natural environment during the summer months.

Although positive net calcification was maintained across all temperature treatments, mean net calcification values suggest water temperature is inversely correlated with net calcification within the temperature ranges used in this experiment (Figure 2). Although the RCP-projected temperature treatments did not exceed normal summer values, coral calcification has been found to have a parabolic response to water temperature, meaning that calcification rates increase with moderate warming, but decrease after a temperature maximum is exceeded (Castillo et al., 2014). This parabolic response may explain why net calcification appears to be negatively correlated with water temperature within the temperature ranges used in this experiment. Since this experiment was conducted under time constraints, the experimental period lasted just four weeks. This condensed experimental period likely did not provide enough time for significant calcification to occur, as calcareous corals grow relatively slowly. Significant and clearer trends in net calcification may result from a longer experimental period.

Initial symbiont density variation may explain colony-level calcification differences

Buoyant weight measurements showed that water temperature affected the net calcification of some colonies but not others (Figure 3). This variation may be explained by the initial tissue color and symbiont density of the colonies, as symbionts provide essential nutrients for calcification (Smith et al., 2016). The experimental colonies varied widely in their initial tissue color and color intensity (Supplemental Figure 5). A previous study found color intensity to be

inversely correlated with chlorophyll density, suggesting corals with low color intensity have high chlorophyll and symbiont density (Winters et al., 2009). Colony A had significantly higher color intensity across all color channels, meaning this colony had significantly less tissue color and symbiont density (Supplementary Figure 5). Colony A also exhibited lower net calcification with increasing water temperature (Figure 3). Colony A ramets in the *in situ* + 6°C (RCP 8.5) temperature treatment were unable to maintain net calcification values consistent with ramets of the same colony in the *in situ* temperature treatment. This may be explained by Colony A's lack of symbionts as an additional nutrient source to support calcification. Conversely, Colony B had lower color intensity across all color channels, meaning this coral had more tissue color and symbiont density (Supplementary Figure 5). Colony B also exhibited similar net calcification independent of water temperature (Figure 3). Colony B's ability to maintain similar net calcification values across all temperature treatments suggest that its high symbiont density may contribute to its unhindered calcification under thermal stress.

Prolonged thermal stress results in loss of tissue color

Change in color intensity and tissue color over time was influenced by water temperature (Figure 4). Ramets in the *in situ* temperature treatment exhibited decreasing intensity values across all color channels throughout the experiment. Intensity values across all colors channels were lower at Week 5 than Week 1, suggesting *in situ* corals became more colorful throughout the experiment (Supplementary Figure 4). Although corals in all temperature treatments initially gained tissue color, corals in the *in situ* + 4°C (RCP 6.0) and the *in situ* + 6°C (RCP 8.5) temperature treatments began losing tissue color by the end of the experimental period (Figure 4). Across all color channels, the intensity of *in situ* + 4°C (RCP 6.0) corals decreased from Week 0 to Week 4, but increased during the final week of the experiment (Supplementary Figure

4). Meaning corals in the *in situ* + 4°C (RCP 6.0) temperature treatment gained tissue color for the first four weeks and lost tissue color in the final week. Ramets in the *in situ* + 6°C (RCP 8.5) temperature treatment exhibited a spike in color intensity across all channels from Week 4 to Week 5, meaning these corals lost a lot of tissue color at the end of the experimental period (Supplementary Figure 4). These trends in color intensity suggest loss of tissue color occurs after prolonged thermal stress.

RGB color analysis is a possible proxy for temperate coral bleaching

Previous studies have found color analysis techniques to accurately assess the symbiont and chlorophyll density of Indo-Pacific corals (Chow et al., 2016; Winters et al., 2009). We will continue to investigate the application of RGB color analysis for a temperate species by performing symbiont counts and comparing symbiont densities to color intensity data. Because our RGB color analysis reflected changes in coral tissue color, we expect this technique to be an accurate method to quantify symbiont density and coral bleaching. We expect high color intensity values (low tissue color) to correlate with low symbiont densities and low color intensity values (high tissue color) to correlate with high symbiont densities.

Confirming RGB color analysis as an accurate proxy for coral bleaching in temperate species is an important step for temperate coral conservation. This method is quick, easy, inexpensive and doesn't harm corals, making it an ideal technique for rapidly assessing coral health both in laboratory experiments and in the field. As anthropogenic climate change continues to increase ocean temperatures and stress corals, rapid and non-destructive methods for assessing coral health, such as color analysis, will become ever more important to predicting the futures of coral species.

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Coral Color Intensity Analysis Utility. Copyright: Alex Blekhman 2005-2008.

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Supplementary Methods

Water temperature

Water temperature within the experimental tanks was controlled by pumping seawater through chillers (AquaEuroUSA Model: MC-1/13HP) with a flow rate of 291 GPH and individual aquarium heaters (Eheim) in each tank. Daily water temperature measurements were recorded using a glass thermometer. A temperature sensor (HOBO Model: Onset U22-001) was placed in one tank of each temperature treatment to monitor the instantaneous temperature in the tanks. The average, daily, manual measurements roughly followed the target temperature regimes set for all three temperature treatments (Supplementary Figure 6).

Salinity

All tanks were maintained at a salinity of 35. Salinity was measured every other day with a refractometer (Cole-Parmer Model: RSA-BR32T). Deionized water was added when necessary to keep water levels constant and maintain salinity levels as evaporation occurred. Three-millimeter-thick Plexiglas sheets were placed on top of each tank to reduce evaporation.

pH, nitrate and flow rate

Tank pH and Nitrate (NO_3^-) concentrations were measured using the Aquarium Pharmaceuticals Saltwater Master Test Kit (API). Tank pH was measured three times per week and was maintained between 8.2 and 8.4. Nitrate concentration was tested two times per week and negligible levels were consistently detected. All three tanks belonging to a particular temperature treatment were connected to the same 190 L sump that circulated water through the tanks. A protein skimmer (Eshopps) removed organic material from the water in the sump. The collection basins of the protein skimmers were cleaned daily. Water flow in the tanks was maintained by pumps (Rio^+ 2100) in each sump at a flow rate of 2630 LPH. Two powerhead pumps (Hydor

USA) were placed in each experimental tank with flow rates of 240 GPH. Fifty-percent water changes were performed twice per week. Water was cooled with a chiller prior to being added to the *in situ* temperature control tanks to avoid a spike in water temperature.

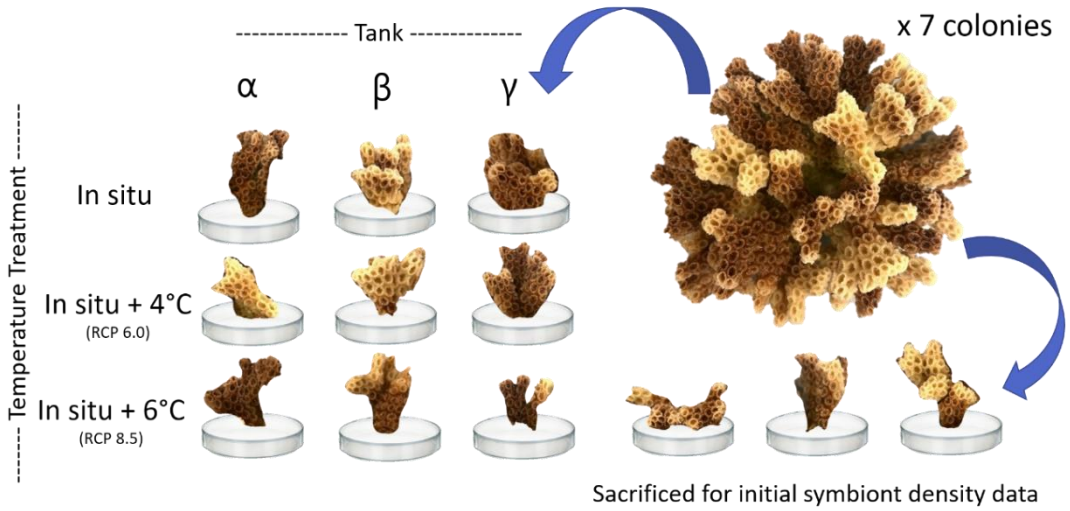
Photosynthetically active radiation

Each tank was illuminated by overhead actinic and daylight bulbs (Hobbie Bug Model: Euphotica Lite 16; frequency 50-60 Hz). The daily light regime was as follows: corals were exposed to white and actinic light for twelve hours and only actinic light for one hour on either end of the twelve-hour period to simulate dawn and dusk. Mesh sheets were placed on the tank lids to replicate light conditions at the collection site. Corals were exposed to an average of 150.1 μmol photons m^{-2} s^{-1} when both the white and actinic lights were on, and an average of 80.6 μmol photons m^{-2} s^{-1} when only the actinic light was on (Supplementary Figure 7).

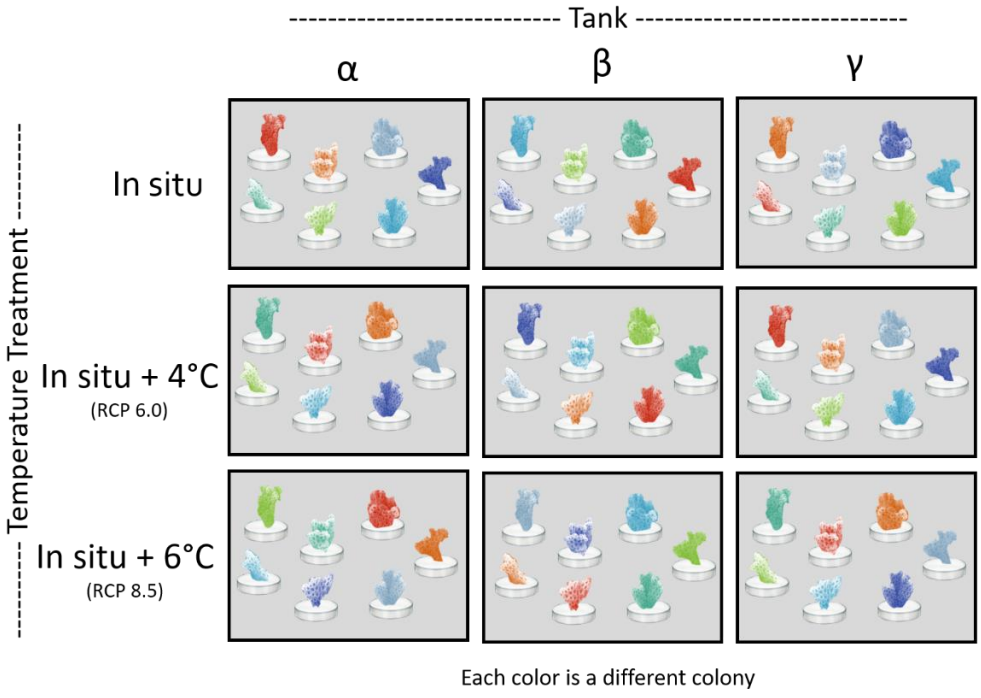
Feeding Regime

To each 38 L tank, 75 mL of approximately 125 *Artemia* per mL was added, resulting in a concentration of 250 *Artemia* per L in each tank. All powerhead pumps were turned off before the *Artemia* was added, and the pumps were turned back on one hour later after giving the corals sufficient time to feed.

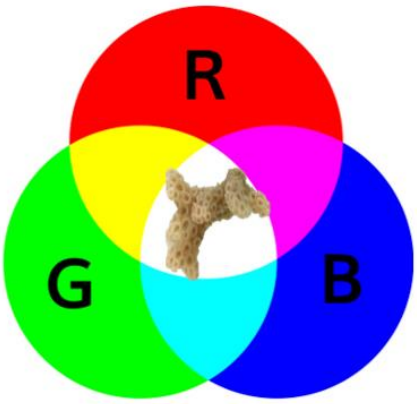
Supplementary Figures



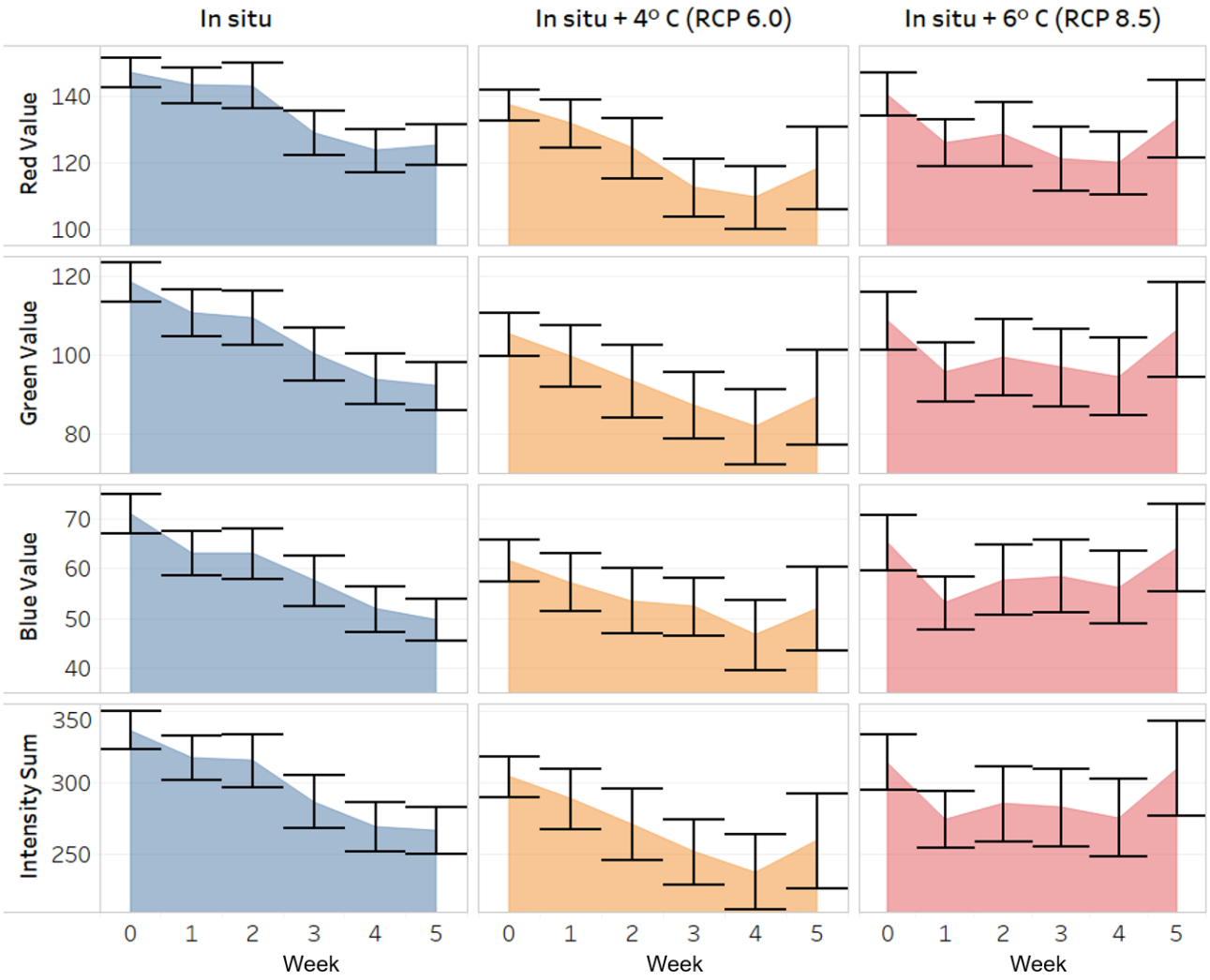
Supplementary Figure 1. Sectioning colonies and assigning ramets to temperature treatment groups. Seven colonies of *O. arbuscula* were sectioned into approximately 5 cm^3 ramets and glued to petri dishes. Each colony was sectioned into twelve ramets, for a total of 84 ramets. Three ramets per colony were sacrificed to obtain initial symbiont density data. Three ramets were assigned to each temperature treatment.



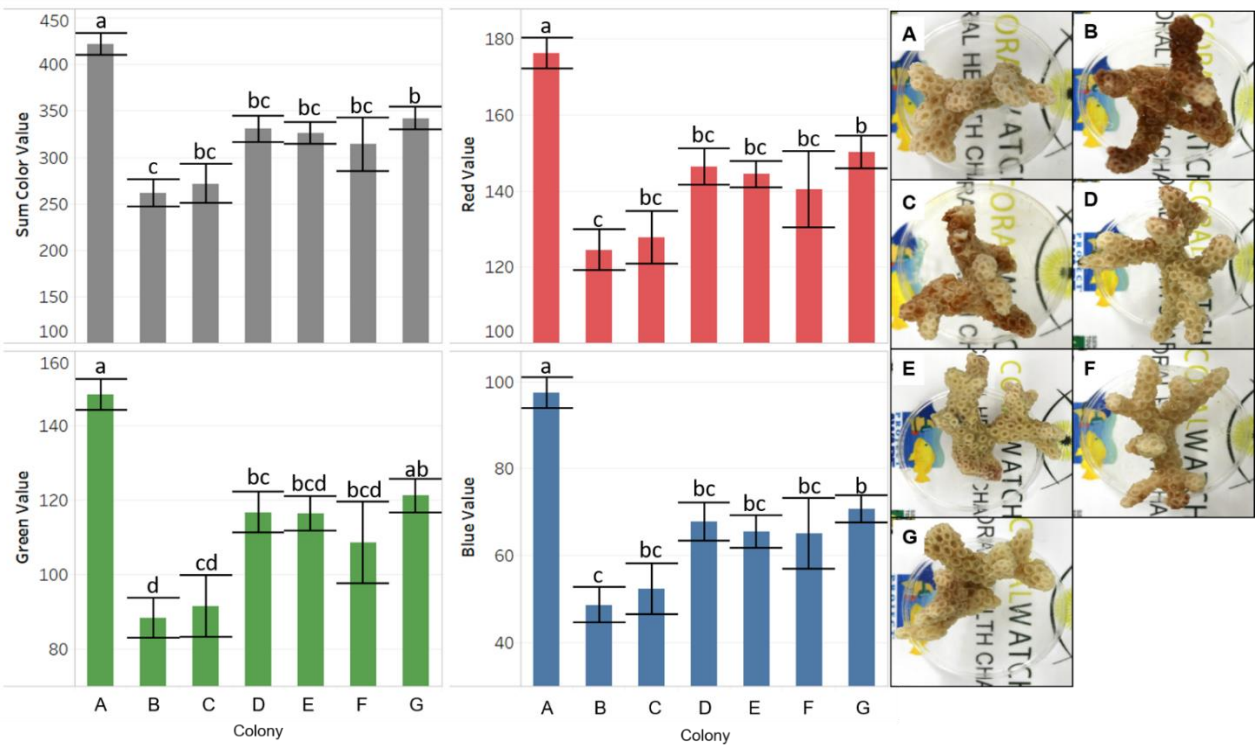
Supplementary Figure 2. Vizualization of ramets in tempeature treatment tanks. Each temperature treatment has three replicate tanks. Each tank contains seven ramets, one from each colony.



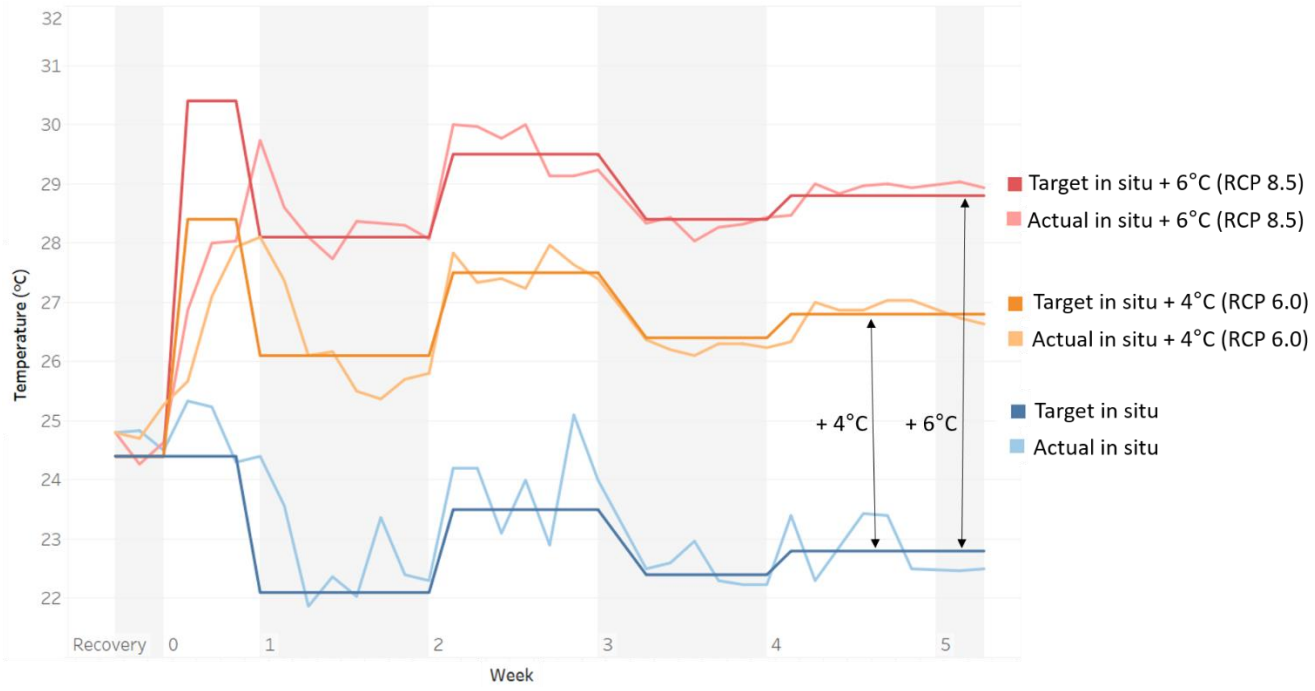
Supplementary Figure 3. RGB color analysis follows additive color mixing. Red, green and blue color channels combine to produce white. Color intensity is inversely proportional to coral tissue color. Corals with low tissue color have high color intensity.



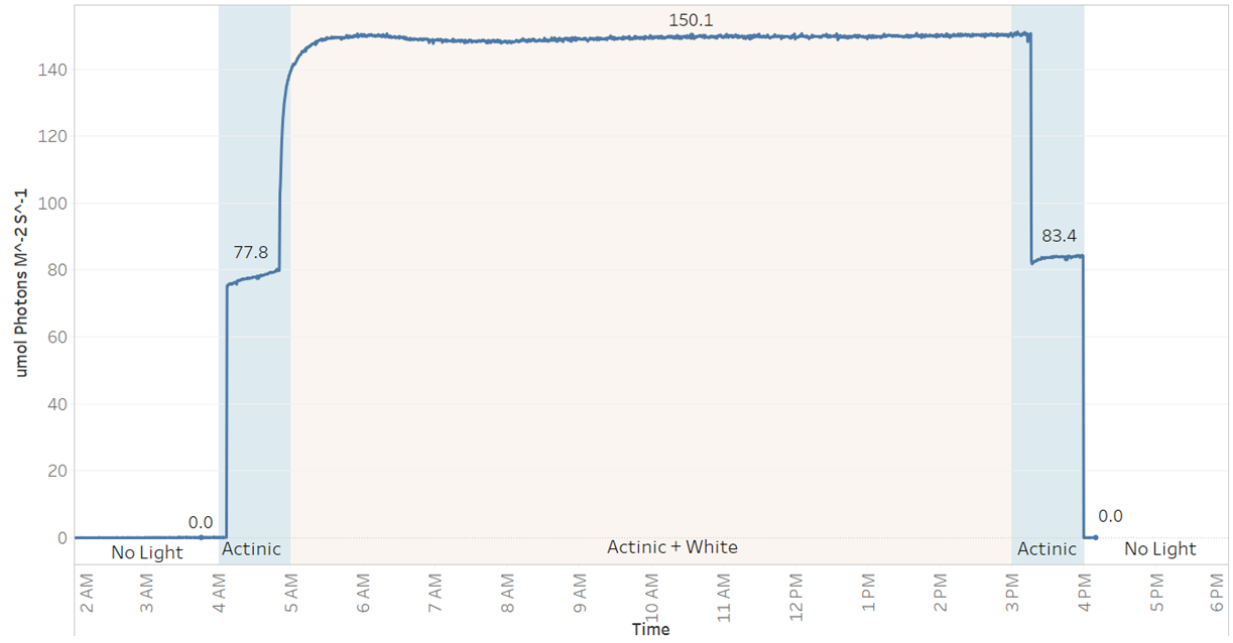
Supplementary Figure 4. The effect of water temperature on red, green, blue and sum color channels across all temperature treatments. Week 0 marks the acclimation period. Week 1 – Week 5 marks the duration of the experimental period. Standard error bars are shown. N = 24 ramets per temperature treatment for Week 0 and 21 ramets per temperature treatment for Week 1- Week 5.



Supplementary Figure 5. Initial (Week 0) color intensity values across all color channels sorted by coral colony. Significant difference groups (Post hoc Tukey HSD) are marked by lowercase letters. Standard error bars are shown. N = 12 ramets per colony. Images show one ramet from each colony during the acclimation period.



Supplementary Figure 6. Daily tank water temperature measurements (actual) compared to target temperature regimes (target) across all temperature treatments. Average temperature in tanks during the expeirmental period: *in situ* (23.4 ± 1.06° C), *in situ* + 4°C/RCP 6.0 (26.5 ± 0.96° C), *in situ* + 6°C/RCP 8.5 (28.15 ± 1.53° C).



Supplementary Figure 7. Daily photosynthetically active radiation regime in experimental tanks. Corals experienced ten hours of daylight intensity (actinic and white light), with one hour of lower intensity (actinic light only) on either end to mimic dawn and dusk light levels. Corals experiened no light during the remaining twelve hours to mimic nightime conditions. Average light intensity for each interval is shown.